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DEPARTMENT OF BIOCHEMISTRY

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Building 1, Room 124
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Dear Don,

Your commentary and the proposed revisions of the Recombinant DNA Guidelines revealed how far we have moved since the issue of potential risks associated with recombinant DNA research was first raised publicly. In spite of the occasional ferocity, stridency and all too frequent politicalization of the discussions, features that turned me and others off to continuing involvement, you and your colleagues have persevered, steered a reasonable course while peppered with foolish and intemperate advice and arrived at proposals, which in the main are workable and a basis for further evaluation.

My estimate of the potential risks of recombinant DNA research and how to deal with them have certainly changed in the last few years. There is nothing remarkable or sinister about that; as scientists we are obliged, constantly, to reexamine and reinterpret our hypotheses, taking into account new developments, new data and fresh insights. The Asilomar Converence Report insisted repeatedly that the premises, conclusions and practices in the recombinant DNA field needed to be reviewed, reevaluated and updated continuously. Lest there be any misunderstanding about how I feel now let me say that I no longer have concerns about the safety of most recombinant DNA experimentation; consequently, it becomes increasingly difficult to justify to myself and others the amount of time and money being expended against risks that are highly improbable: perhaps more unlikely than the probability that space vehicles reentering our atmosphere will fall in populated areas (a possibility which elicited little concern or comment from the media or public spirited citizens) or any one of hundreds of other potentially calamitous but routine activities and practices. I could be mistaken in my estimate of the risks but I don't believe so. However, being fallible, I have supported the NIH's efforts to devise sensible and workable guidelines to minimize the possibility of any risk. I can continue to do so as long as they do not become unnecessarily restrictive and stifling to research progress

and creativity. Unfortunately, some portions of The Guidelines, but particularly the implementation of The Guidelines, are already having that effect. I urge you to be on guard against that outcome.

I have from time to time reflected on my participation in raising the recombinant DNA issue in 1974. Considering what we knew then and what we perceived as the risks, I don't see how I or any of us could have done otherwise. Had we been privy to the information, discussions and experience available today, that action would surely not have been taken. Opponents of recombinant DNA research, or those wishing to restrict its applications, can no longer cite that document as an expression of present day concerns.

There are several specific comments about PRG-NIH that I want to register; hopefully they will be helpful to you in arriving at the final form of this version of The Guidelines.

1. Your decision to remove a large number of innocuous experiments from the purview of The Guidelines is a wise one; such experiments have never been the subject of concern even amongst those who opposed the research. I am also in general agreement with the reduction in containment requirements for most experiments using the EK host-vector systems. The large body of experience gained during the past four years and the advice of infectious disease experts, epidemiologists and evolutionists suggests that the earlier concerns were unwarranted.

2. Earlier I had suggested that PRG-RAC's recommendations for experimentation with recombinants containing animal or plant virus DNAs needed to be reevaluated. The present recommendations, drawn from the US-EMBO workshop and review committee's reports, are more realistic, reflecting a different assessment of the risk-benefit equation than was made four years ago. This line of experimentation should lead to important advances in understanding viral genome structure and function. In my view the potential benefits of permitting these experiments to proceed as now recommended far outweigh the hypothetical and rather unlikely risks; consequently, I support the recommendations in Section III-A-2.

3. As I am involved in such research I'd like to comment at greater length on the containment requirements for experiments using animal virus vectors for cloning in mammalian cells (Section-C).

a) Infection of non-permissive cells results in a non-productive infection, but non-productive infections may also result from infection of <u>permissive cells</u> e.g. when

permissive cells are infected with mutant genomes which can not complete the infectious cycle. I foresee the development of eukaryote plasmids constructed from portions of Polyoma or SV40 DNA that can propagate in permissive mammalian cells without killing the cells or producing progeny virus. That contingency is obscured by using the words non-permissive cells in connection with non-productive infections.

b) I am totally confused by the wording or intent of paragraphs III-C-1-a-(1)-(b and c) dealing with polyoma vectors particularly when they are compared with the analagous instructions for the SV40 DNA vectors in paragraph III-C-1-b-(1)-(b). The word defective and intact are not appropriate. How can one use an intact polyomaDNA as a vector? If an exogenous DNA sequent is introduced into the polyoma DNA it is no longer intact; moreover, the introduction of foreign DNA into the polyoma DNA will render it defective because it will inactivate an essential function or enlarge the DNA to a size that prevents its encapsidation-hence it becomes defective as a potential virus. I think The Guidelines want to distinguish between the cases where a recombinant is defective, therefore requiring a helper, and the very rare case where a recombinant is non-defective and does not need a helper for multiplication. The operational definitions that define the containment requirements should be whether the propagation of the recombinant is helper-dependent or independent.

c) In assigning P2 containment to experiments in which african green monkey cell DNA is propagated with SV40 DNA vectors, were human and rhesus monkey cell DNA to be excluded from this category? Human and some other primate cells do support SV40 multiplication and, therefore, presumably cell-virus DNA recombination does occur naturally; shouldn't paragraph III-C-1-b-(a)-(2) read "uninfected primate cells in which SV40 is known to multiply"? I am also puzzled by the logic which permits P2 containment for propagating monkey DNA in SV40 vectors but retains a P3 containment requirement for propagating recombinants with yeast, Drosophila or rabbit DNA (compare paragrahs III-C-1-b-(1)-(a)-(2) and III-C-1-b-(1)-(b).

4. Another point which needs further consideration is the continued use and definition of the words "purified" and "vigorously characterized" (see paragraph III-A). In as much as I drafted the wording to explain the terms appearing in footnote 38 and 40 of Section V at the RAC meeting in La Jolla in December 1975, I know that its adoption reflected

the supercautious mood prevalent at that time rather than a clear-cut scientific judgement. In my view requiring that a DNA fragment should be 90-95% pure (as judged by at least two different analytical procedures) to remove it from the "shotgun" classification is more realistic and no less safe. Requiring that a DNA fragment be >99% pure prior to cloning asks for the most stringent and detailed documentation without any real advantage. For example, if prokaryote or mouse DNA, can be "shotgunned" into polyoma vectors under P2 conditions, I do not understand why segments of DNA from eukaryotes e.g. or gamisms that do not produce potent polypeptide toxins as exemplified by yeast, Drosophila or even rabbit, need be >99% pure prior to cloning in polyoma! What is the concern if they were 50, 90 or 95% pure?

In the paragraphs of Section III-A-2, dealing with cloning various virus DNA and cDNA sequences, the word purified appears throughout without referencing to footnote 38 or 40. Does purified in each of these cases mean >99%? If so, is that intentional or inadvertent? In my view if a particular segment was 90-95% pure that would be sufficient since the isolation and screening of only 20 to 50 clones would insure the recovery of the desired recombinant with little or no increased risk. Even with DNA segments that are 50% pure half the clones would be the desired ones. In short by recommending that segments be greater than 90% pure only a small number of clones need to be isolated and examined, thereby, drastically reducing the probability of creating or releasing unexpected recombinants to a vanishingly small number.

5. I am particularly unhappy about the recommendations PRG-NIH proposes regarding the development, review and certification of new HV systems. The breakthrough with yeast transformation and the ability to propagate exogenous DNAs in S. cerevesiae has very important ramifications in extending the recombinant DNA technique for basic and applied purposes. Yet ORDA and RAC are confounding and, I believe obstructing these developments. Long delays in coming to a conclusion are particularly frustrating. I suggest that as such scientific problems arise ad hoc committees, composed of knowledgeable and responsible scientists in the relevant disciplines, should be convened as quickly as possible to examine the issues and advise RAC on possible courses of action. The U.S.-EMBO workshop and report is a model of how this can be done. In this way actions on several important issues could be proceeding in parallel rather in series. Such committees or workshops should already be at work trying to assess the possible risks, if any, of cloning foreign DNAs in yeast and in B. subtilus. As it now stands no decision is tantamount to a ban on these experiments. Can we really defend or condone banning the introduction of Drosophila or mammalian DNAs into S. cervesiae? Even with the uncertainties that existed at Asilomar cloning such DNAs in E. coli K12 was not forbidden. While the inquiries are proceeding there should be recommendations of interim physical containment and specified biological properties to permit such experiments to proceed. Investigators would be informed

that such recommendations are tentative and will be modified as new information becomes available. This would permit investigators to explore the feasibility of such experiments and lay the groundwork for settling on the final containment requirements of such experiments. As matters now stand the absence of a policy is tantamount to a ban on such experiments.

6. There are also several comments and concerns about the procedures set forth in PRG-NIH.

a) I support enthusiastically the change that permits local IBC's to approve proposals and the investigator to initiate the experiments after obtaining such approval. The previous procedures were cumbersome, time-consuming and unnecessary. In my view the greatest risk to non-compliance with The Guidelines is for investigators to be confronted with an unresponsive, time-wasting bureaucracy. I have been impressed with how efficiently and unobtrusively (for the institutions and investigators) the procedures for safe-handing of radio-isotopes are administered. Hopefully surveillance of recombinant DNA research can be made as routine as the procedures used for experimentation with radio isotopes.

b) As a member of an IBC I am particularly sensitive to the workload imposed on that body by the Recombinant DNA Guidelines. I am sure you are cognizant of the monetary costs in faculty and staff time needed to enforce them. Presumably, this gets charged to indirect costs, thereby making the costs of research to all granting agencies still higher. I hesitate to inquire what the costs of IBC time have been to Stanford during the past year. I suspect that these costs together with those attributable to your interminable involvement in these matters introduces a further and substantial drain on an already shrinking NIH research budget.

c) I note that the PRG-NIH requires that all, not just NIH funded, recombinant DNA research at institutions receiving NIH support must comply with The Guidelines. But the instructions for how non-NIH funded research is to be reviewed, reported, monitored etc. are not clear enough. My deductions from reading Section IV A-D in Appendix C (particularly paragraphs IV D 1-10) are that a non-NIH funded project must be reviewed, approved and reported as is done for NIH-supported research. Since formal approval is no longer needed prior to initiating the work, what is the difference between the two procedures? Why couldn't the instructions read, that all recombinant DNA research, regardless of its funding source but carried out in an institution receiving NIH funds, must be treated as if the research is NIH-funded? In that way

one procedure would do for all? It would greatly ease the burden of the IBC's to treat all recombinant DNA projects alike.

7. I seriously question the necessity for public discussions and review of each and every subsequent modification in the Guidelines and its Appendices. I believe the process is already public enough in as much as the Director's Advisory Committee and RAC have public members and meet in full public view, only after public announcement of the meeting's time and place; changes in the Guidelines are made in close consultation with these groups and final decisions are published in the Federal Register and Recombinant DNA Bulletin. Must every technical decision be the subject of the same interminable review process now in progress? I was struck by the number of places in PRG-NIH where the Director is required to provide "appropriate notice and opportunity for public comment" prior to making or implementing decisions. I believe a distinction should be made between purely technical and scientific matters and broader policy issues that frequently elicit political, ethical, religious and other considerations.

8. The phrase "case by case" review occurs frequently in PRG-NIH, particularly where construction of recombinants involving two different viral DNAs is considered. But I found nothing more than general statements about how such case by case review would be conducted. May I suggest that an ad hoc committee or subcommittee of RAC formulate a plan for how such reviews will be conducted e.g., by whom (ORDA or RAC?), when (on demand or periodically), what constitutes a decision and who makes it (the Director, ORDA or RAC?) etc. Will such decisions also require public review and comment? That group could also construct a list of putative high, intermediate - and low-risk combinations to guide investigators now planning such experiments. For example, there is considerable interest for introducing the thymidine kinase gene from herpes simplex virus into either SV40 or polyoma DNA vectors; this is quite a straight-forward almost trivially simple, but nevertheless important experiment; yet, presently it is a forbidden experiment and unless the case by case reviews are expedited it will remain forbidden.

I had not intended to write at such length but my justification for doing so is that you might find my comments useful in your deliberations.

This summer as I sat through two Gordon Conferences reviewing the recent advances using the recombinant DNA methods I wished that you were there. I particularly wanted you to see and experience, first hand, the dynamism, excitement and explosive progress that these developments have engendered. The recombinant DNA methodology and rapid nucleic acid sequencing

techniques have revolutionized molecular biology and genetic chemistry; the ignorance and confusions that obscure and delay solutions to important biomedical problems are being blown aside. I am more than ever confident that we, you, I and the many others who have had a hand in initiating and supporting recombinant DNA research, will live to see its fruits harvested.

With sincere best wishes for continued success,

Sincerely,

Paul

Paul Berg

PB:vs